

PCT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner
 US Department of Commerce
 United States Patent and Trademark
 Office, PCT
 2011 South Clark Place Room
 CP2/5C24
 Arlington, VA 22202
 ETATS-UNIS D'AMERIQUE
 in its capacity as elected Office

Date of mailing (day/month/year) 13 December 2000 (13.12.00)	
International application No. PCT/NL00/00200	Applicant's or agent's file reference BO 42517
International filing date (day/month/year) 24 March 2000 (24.03.00)	Priority date (day/month/year) 24 March 1999 (24.03.99)
Applicant DE WINTER, Edwin, Johannus, Gerardus et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:
 18 October 2000 (18.10.00)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was

☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Pascal Piriou
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

JORRITSMA, Ruurd
Nederlandsch Octrooibureau
Scheveningseweg 82
P.O. Box 29720
NL-2502 LS The Hague
PAYS-BAS

Date of mailing (day/month/year) 26 October 2001 (26.10.01)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference BO 42517	
International application No. PCT/NL00/00200	International filing date (day/month/year) 24 March 2000 (24.03.00)

1. The following indications appeared on record concerning:	
<input checked="" type="checkbox"/> the applicant	<input type="checkbox"/> the inventor <input type="checkbox"/> the agent <input type="checkbox"/> the common representative
Name and Address INSTITUUT VOOR AGROTECHNOLOGISCH ONDERZOEK (ATO-DLO) P.O. Box 17 NL-6700 AA Wageningen Netherlands	State of Nationality NL
	State of Residence NL
	Telephone No.
	Facsimile No.
2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:	
<input checked="" type="checkbox"/> the person	<input type="checkbox"/> the name <input type="checkbox"/> the address <input type="checkbox"/> the nationality <input type="checkbox"/> the residence
Name and Address ATO B.V. Bornsesteeg 59 NL-6708 Wageningen Netherlands	State of Nationality NL
	State of Residence NL
	Telephone No.
	Facsimile No.
3. Further observations, if necessary:	
4. A copy of this notification has been sent to:	
<input checked="" type="checkbox"/> the receiving Office	<input type="checkbox"/> the designated Offices concerned
<input type="checkbox"/> the International Searching Authority	<input checked="" type="checkbox"/> the elected Offices concerned
<input type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:

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AUG 7 - 2002
TC 1700

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Idhir BRITEL
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

P A T E N T COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

JORRITSMA, Ruurd
Nederlandsch Octrooibureau
Scheveningseweg 82
P.O. Box 29720
NL-2502 LS The Hague
PAYS-BAS

Date of mailing (day/month/year)
28 August 2001 (28.08.01)

Applicant's or agent's file reference
BO 42517

International application No.
PCT/NL00/00200

IMPORTANT NOTIFICATION

International filing date (day/month/year)
24 March 2000 (24.03.00)

1. The following indications appeared on record concerning:

☒ the applicant ☒ the inventor ☐ the agent ☐ the common representative

Name and Address

BARTELS, Paul, Vincent
Graspieper 23
NL-6708 LR Wageningen
Netherlands

State of Nationality

NL

State of Residence

NL

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address

BARTELS, Paul, Vincent
Graspieperweide 23
NL-6708 LR Wageningen
Netherlands

State of Nationality

NL

State of Residence

NL

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office ☐ the designated Offices concerned
☐ the International Searching Authority ☒ the elected Offices concerned
☒ the International Preliminary Examining Authority ☐ other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Anman QIU

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

JORRITSMA, Ruurd
Nederlandsch Octrooibureau
Scheveningseweg 82
P.O. Box 29720
NL-2502 LS The Hague
PAYS-BAS

Date of mailing (day/month/year) 20 September 2001 (20.09.01)
Applicant's or agent's file reference BO 42517
International application No. PCT/NL00/00200

IMPORTANT NOTIFICATION
International filing date (day/month/year) 24 March 2000 (24.03.00)

1. The following indications appeared on record concerning: <input checked="" type="checkbox"/> the applicant <input checked="" type="checkbox"/> the inventor <input type="checkbox"/> the agent <input type="checkbox"/> the common representative		
Name and Address DE WINTER, Edwin, Johannus, Gerardus Tarthorst 133 NL-6708 HG Wageningen Netherlands	State of Nationality NL	State of Residence NL
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	
2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning: <input type="checkbox"/> the person <input type="checkbox"/> the name <input checked="" type="checkbox"/> the address <input type="checkbox"/> the nationality <input type="checkbox"/> the residence		
Name and Address DE WINTER, Edwin, Johannus, Gerardus Zandstraat 96 NL-4101 EJ Culemborg Netherlands	State of Nationality NL	State of Residence NL
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	
3. Further observations, if necessary:		
4. A copy of this notification has been sent to: <input checked="" type="checkbox"/> the receiving Office <input type="checkbox"/> the designated Offices concerned <input type="checkbox"/> the International Searching Authority <input checked="" type="checkbox"/> the elected Offices concerned <input type="checkbox"/> the International Preliminary Examining Authority <input type="checkbox"/> other:		

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Ingrid AULICH Telephone No.: (41-22) 338.83.38
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PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

JORRITSMA, Ruurd
Nederlandsch Octrooibureau
Scheveningseweg 82
P.O. Box 29720
NL-2502 LS The Hague
PAYS-BAS

Date of mailing (day/month/year) 20 September 2001 (20.09.01)
Applicant's or agent's file reference BO 42517
International application No. PCT/NL00/00200

IMPORTANT NOTIFICATION
International filing date (day/month/year) 24 March 2000 (24.03.00)

1. The following indications appeared on record concerning:		
<input checked="" type="checkbox"/> the applicant	<input checked="" type="checkbox"/> the inventor	<input type="checkbox"/> the agent <input type="checkbox"/> the common representative
Name and Address DE WINTER, Edwin, Johannus, Gerardus Tarthorst 133 NL-6708 HG Wageningen Netherlands	State of Nationality NL	State of Residence NL
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	
2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:		
<input type="checkbox"/> the person	<input type="checkbox"/> the name	<input checked="" type="checkbox"/> the address <input type="checkbox"/> the nationality <input type="checkbox"/> the residence
Name and Address DE WINTER, Edwin, Johannus, Gerardus Zandstraat 96 NL-4101 EJ Culemborg Netherlands	State of Nationality NL	State of Residence NL
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	
3. Further observations, if necessary:		
4. A copy of this notification has been sent to:		
<input checked="" type="checkbox"/> the receiving Office	<input type="checkbox"/> the designated Offices concerned	
<input type="checkbox"/> the International Searching Authority	<input checked="" type="checkbox"/> the elected Offices concerned	
<input type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:	

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Ingrid AULICH
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference BO 42517	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/NL 00/ 00200	International filing date (day/month/year) 24/03/2000	(Earliest) Priority Date (day/month/year) 24/03/1999
Applicant INSTITUUT VOOR AGROTECHNOLOGISCH ONDERZOEK (ATO-DL		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.
☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
- ☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :
- ☐ contained in the international application in written form.
 - ☐ filed together with the international application in computer readable form.
 - ☐ furnished subsequently to this Authority in written form.
 - ☐ furnished subsequently to this Authority in computer readable form.
 - ☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
 - ☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the title,

- ☐ the text is approved as submitted by the applicant.
- ☒ the text has been established by this Authority to read as follows:

METHOD FOR TREATING PRODUCTS BY HIGH VOLTAGE PULSES

5. With regard to the abstract,

- ☒ the text is approved as submitted by the applicant.
- ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

- ☐ as suggested by the applicant.
- ☒ because the applicant failed to suggest a figure.
- ☐ because this figure better characterizes the invention.

2B _____

☐ None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No

NL 00/00200

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 A23L3/32 A23B5/015

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A23L A23B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, PAJ, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LUBICKI P ET AL: "INACTIVATION OF YERSINIA ENTEROCOLITICA GRAM-NEGATIVE BACTERIA USING HIGH VOLTAGE PULSE TECHNIQUE", RECORD OF THE INDUSTRY APPLICATIONS CONFERENCE (IAS), ORLANDO, OCT. 8 - 12, 1995, VOL. 2, NR. CONF. 30, PAGE(S) 1338 - 1344, INSTITUTE OF ELECTRICAL AND ELECTRONICS ENGINEERS XP000546877 ISBN: 0-7803-3009-9 page 1339, column 1, line 3 -column 2, line 11; figures 4,2,1 ---	1,4-7,10
P,X	US 6 027 754 A (BUSHNELL ANDREW H ET AL) 22 February 2000 (2000-02-22) claims 1,15-17 --- -/--	1,4,5,8, 10

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

28 June 2000

Date of mailing of the international search report

18/07/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 340-3016

Authorized officer

Guyon, R

INTERNATIONAL SEARCH REPORT

International Application No

NL 00/00200

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 690 978 A (YIN YONGGUANG ET AL) 25 November 1997 (1997-11-25) cited in the application column 10, line 61 -column 11, line 11; claims 17,20 column 13, line 6 - line 34 column 11, line 30 -column 12, line 33 ---	1-5,8-10
X	US 5 776 529 A (B.L. QIN ET AL.) 7 July 1998 (1998-07-07) column 12, line 40 -column 13, line 4; figures 13-19; examples 13-19 column 6, line 53 -column 7, line 8 ---	1-10
A	US 4 838 154 A (J.E. DUNN ET AL.) 13 June 1989 (1989-06-13) column 6, line 45 - line 65; claims 1,4 ---	1-10
X	US 4 695 472 A (J.E.DUNN ET AL.) 22 September 1987 (1987-09-22) column 6, line 35 - line 55; claims 1,15,16 column 6, line 64 -column 7, line 15 ---	1-10
A	B.L. QIN ET AL.: "Inactivating microorganisms using a pulsed electric field continuous treatment system" IEEE TRANSACTIONS ON INDUSTRY APPLICATIONS, vol. 34, no. 1, 1 February 1998 (1998-02-01), pages 43-49, XP000766888 the whole document ---	1
A	US 5 235 905 A (A. H. BUSHNELL ET AL.) 17 August 1993 (1993-08-17) column 10, line 56 -column 12, line 50 column 12, line 64 -column 13, line 15 ---	1-10
A	US 5 447 733 A (A.H.BUSHNELL ER AL.) 5 September 1995 (1995-09-05) cited in the application claim 1; figures 6A,6B,13 ---	1
X	US 5 549 041 A (ZHANG QINGHUA ET AL) 27 August 1996 (1996-08-27) column 7, line 6 - line 23 ---	1-10
X	WO 93 25097 A (FOODCO CORP) 23 December 1993 (1993-12-23) the whole document ---	1-10
X	US 5 514 391 A (BUSHNELL ANDREW H ET AL) 7 May 1996 (1996-05-07) column 11, line 46 - line 65; claims 1,6 ---	1,4,5,8

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INTERNATIONAL SEARCH REPORT

International Application No

NL 00/00200

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 38045 A (KNIPPER ALOYSIUS J ;POLNY THADDEUS J JR (US)) 5 December 1996 (1996-12-05) claims 7-9 -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

NL 00/00200

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 6027754	A	22-02-2000	AU 3772599 A WO 0000044 A	17-01-2000 06-01-2000
US 5690978	A	25-11-1997	AU 4605697 A WO 9814074 A	24-04-1998 09-04-1998
US 5776529	A	07-07-1998	US 5662031 A US 6019031 A	02-09-1997 01-02-2000
US 4838154	A	13-06-1989	US 4695472 A US 5235905 A EP 0340212 A WO 8803763 A US 5048404 A	22-09-1987 17-08-1993 08-11-1989 02-06-1988 17-09-1991
US 4695472	A	22-09-1987	EP 0340212 A US 4838154 A WO 8803763 A US 5048404 A US 5235905 A	08-11-1989 13-06-1989 02-06-1988 17-09-1991 17-08-1993
US 5235905	A	17-08-1993	US 4838154 A US 5048404 A US 4695472 A AU 669725 B AU 4527693 A CA 2135936 A EP 0642309 A JP 8500968 T WO 9325097 A AU 640384 B AU 5836190 A CA 2057031 A,C DE 69033273 D DE 69033273 T EP 0594566 A ES 2135379 T JP 4506151 T WO 9015547 A EP 0340212 A WO 8803763 A	13-06-1989 17-09-1991 22-09-1987 20-06-1996 04-01-1994 23-12-1993 15-03-1995 06-02-1996 23-12-1993 26-08-1993 08-01-1991 13-12-1990 07-10-1999 30-12-1999 04-05-1994 01-11-1999 29-10-1992 27-12-1990 08-11-1989 02-06-1988
US 5447733	A	05-09-1995	US 5393541 A AU 1557395 A CA 2180159 A EP 0738116 A JP 9510867 T WO 9518548 A	28-02-1995 01-08-1995 13-07-1995 23-10-1996 04-11-1997 13-07-1995
US 5549041	A	27-08-1996	NONE	
WO 9325097	A	23-12-1993	US 5235905 A AU 669725 B AU 4527693 A CA 2135936 A EP 0642309 A JP 8500968 T	17-08-1993 20-06-1996 04-01-1994 23-12-1993 15-03-1995 06-02-1996

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/00200

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5514391 A	07-05-1996	AU 5183396 A	30-12-1996
		CA 2223509 A	19-12-1996
		EP 0831729 A	01-04-1998
		JP 11506011 T	02-06-1999
		WO 9639876 A	19-12-1996
WO 9638045 A	05-12-1996	US 5741539 A	21-04-1998

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference BO 42517	<div style="display: flex; justify-content: space-between;"> <div> FOR FURTHER ACTION </div> <div> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) </div> </div>	
International application No. PCT/NL00/00200	International filing date (day/month/year) 24/03/2000	Priority date (day/month/year) 24/03/1999
International Patent Classification (IPC) or national classification and IPC A23L3/32		
Applicant INSTITUUT VOOR AGROTECHNOLOGISCH ONDERZOEK (ATO-DL)		
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 8 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 2 sheets.</p>		
<p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input checked="" type="checkbox"/> Certain documents cited VII <input checked="" type="checkbox"/> Certain defects in the international application VIII <input checked="" type="checkbox"/> Certain observations on the international application 		
Date of submission of the demand 18/10/2000	Date of completion of this report 14.05.2001	
Name and mailing address of the international preliminary examining authority: <div style="display: flex; align-items: center;"> <div> European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465 </div> </div>	Authorized officer Diez Schlereth, D Telephone No. +49 89 2399 7488	



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL00/00200

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, pages:

1-13 as originally filed

Claims, No.:

1-7 as received on 13/03/2001 with letter of 13/03/2001

Drawings, sheets:

1/1 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL00/00200

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims 1-7
	No: Claims
Inventive step (IS)	Yes: Claims 1-7
	No: Claims
Industrial applicability (IA)	Yes: Claims 1-7
	No: Claims

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/NL00/00200

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/NL00/00200

item V.

1.) Reference is made to the following documents:

D1: US-A-5,690,978

D2: US-A-5,776,529

D3: US-A-4,695,472

D4: P. Lubicki et al., IEEE, IAS Conference, Orlando 8-12 Oct. 1995, vol. 2, No 30, 1338-1344

D5: US-A-5,549,041

D6: US-A-5,514,391

D7: WO-A-93/25097

2.) The subject-matter of claim 1 (and 2-7 as dependent thereon) is considered to be novel and inventive within the sense of Art. 33 (2) and (3) PCT, for the following reasons:

D1, which is considered to be the closest prior art, discloses a device (and a method) for sterilization and preservation of pumpable food products, which comprises two electrodes, each electrode including an electrode flow chamber for making electrical contact with the food product and for allowing the product to flow through the device. Food products are treated by applying high voltage pulses of variable voltage (electrical field strength between 15-160 kV/cm), frequency (20-500 Hz) and pulse duration (1-20 microseconds), see col. 1, l. 10-15; col. 2, l. 5-67; col. 3, l. 1-37.

D2 discloses a method for inactivating microbes in flowable food products by applying a bipolar pulsed high voltage (10-100 kV) at a frequency between 20-2000 Hz, and with a pulse duration of 0.1-100 microseconds (see col. 1, l. 20-25; col. 6, l. 9-23, 53-67).

D3 discloses a method for preserving fluid food products by applying a controlled pulsed high voltage (electrical field strength between 5-25 kV/cm) at a frequency between 0.1-100 Hz, and with a pulse duration of 1-100 microseconds (see col. 1, l. 5-12; col. 5, l. 10-40; col. 6, l. 35-68).

D4 discloses a method for causing the irreversible electroporation of the bacteria

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/NL00/00200

Yersinia enterocolitica by applying high voltage pulses of peak voltage of 5-75 kV, at a frequency of 1 Hz, and with a rise time of 0.5-1.3 microseconds (Abstract, p. 1341-1343).

D5 discloses a method for inhibiting or preventing microbial growth in solid or semi-solid food products by applying a pulsed high voltage (electrical field strength between 20-100 kV/cm) at a frequency of 1-100 Hz, and with a pulse duration of 0.1-5 microseconds (see col. 1, l. 5-10; col. 2, l. 1-6; col. 7, l. 5-25; col. 9, l. 35-67; col. 10, l. 1-61).

D6 discloses a method for reducing levels of microorganisms in pumpable food products by applying a pulsed high voltage (electrical field strength of 10-120 kV/cm) with a pulse duration of 0.1-20 microseconds in a plurality of electric field treatment zones of said product (see col. 1, l. 9-33; col. 3, l. 54-67; col. 4, l. 1-38; col. 5, l. 42-45; col. 8, l. 18-45; col. 9, l. 1-10; col. 11, l. 47-67).

D7 discloses a method for extending the shelf life of pumpable food products by applying a pulsed high voltage (electrical field strength of 25-120 kV/cm) with a pulse duration of 0.1-25 microseconds through all of the foodstuff (see p. 1, l. 14-22; p. 7, l. 28-35; p. 10, l. 14-37; p. 11, l. 1-16; p. 21, l. 33-37; p. 22, l. 1-12).

The subject-matter of claim 1 differs from the closest prior art (D1) in that the **rise time** or the leading edge **of each imposed voltage pulse is shorter than the associated electronic relaxation time of the product** under treatment, which is defined by the ratio of electrical conductivity and permittivity of the product.

In the light of the closest prior art (D1), the technical problem to be solved by the present application was to provide an alternative method for mild preservation of food products.

The method of claim 1 shows the following advantages over other methods known from the prior art: (i) it can be used as a preservation method for prepacked products, since it does not require a physical contact of the electrodes with the product, (ii) it is faster because the duration of the pulses can be very small (as the biocide effect arises from the intensity of the voltage during the rise time of the pulse which is shorter than the

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relaxation time of the product), and (iii) because the voltage is applied during a very short time the temperature increase during the process is very small.

The methods for preserving food disclosed in D1-D3 and D5-D6 are based on the application of pulses of voltage during a certain time. None of these documents mentions neither the duration of the rise time of the pulse, nor the possible influence of said rise time in the biocide effect of the methods. D4 explicitly discloses the use of high voltages with a rise time of 0.5 to 1.3 microseconds to electroporate irreversibly bacterial cell membranes. The document does not indicate a possible practical application to the method for preserving food products, and more importantly, the used rise times are much larger than the relaxation time of bacteria (3-5 ns). The method for preserving food products disclosed in D7 is based on the same principles than those of D1-D3 and D5-D6. From Figs. 10-15 of this document, however, it can be estimated that a rise time of 500 ns is used. This value, is much higher than the relaxation time of the products treated (between 0.5 to 8.9 ns).

It would appear that none of documents D1-D7 discloses or suggests the use of a series of high voltage pulses in which the duration of the rise time of each imposed voltage pulse is shorter than the relaxation time of the product under treatment for inhibiting or preventing microbial growth.

item VI.

Certain published documents (Rule 70.10)

Application No Patent No	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim) (day/month/year)
US-A-6,027,754	22.02.2000	30.06.1998	-----

This document discloses a method for deactivating microorganisms in food products by applying a pulsed high voltage (electrical field strength of 5-20 kV/cm) with a pulse duration of 2-100 microseconds while flowing the product through a treatment zone (see col. 1, l. 5-13; col. 2, l. 42-46; col. 4, l. 9-41; col. 5, l. 25-62).

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item VII.

Contrary to the requirements of Rule 5.1 (a) (ii) PCT, the relevant background art disclosed in the documents D5-D7 is not mentioned in the description, nor are these documents identified therein.

item VIII.

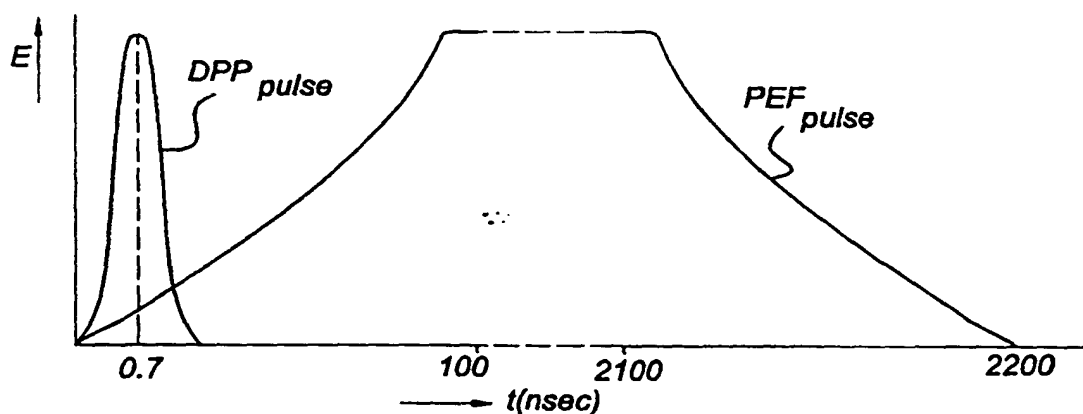
- 1.) The description is not adapted to the wording of the claims (Art. 6 PCT).
- 2.) There are two claims 7 (Art. 6 PCT).



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(54) Title: METHOD FOR TREATING PRODUCTS BY HIGH VOLTAGE PULSES



(57) Abstract

The invention relates to a method for treating products, which may contain cellular material of eukaryotic and/or prokaryotic origin and in particular micro-organisms, by bringing the product in device comprising two electrodes connected to an electronic circuit such that in said device and in said product a pulsating electrical field is created, characterised by the rise time of each imposed voltage pulse which is less than the electronic relaxation time of the product. Preferably the rising edge of each cycle starts within the relaxation time of the product and even more preferably each electrical field pulse has a duration shorter than the relaxation time of the product. Dependent on the type of product and the types of micro-organisms contained in the product, the maximum fieldstrength of each pulse, the repetition frequency and the number of cycles in the treatment are selected such that the target micro-organisms and/or spores are functionally inactivated leading to a shelf stable, microbiologically safe product.

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METHOD FOR TREATING PRODUCTS BY HIGH VOLTAGE PULSES

INTRODUCTION

The invention relates to a method for treating products, which may contain
5 cellular material of eukaryotic and or prokaryotic origin, in particular micro-organisms,
located in a device comprising two electrodes onto where voltage cycles are imposed
by an auxiliary electric source such that in the device and in the product electrical fields
are created for a short period of time.

A prior art method of this type is known as the Pulsed Electric Field (PEF)
10 process.

PRIOR ART

Many biological systems, such as micro-organisms, comprise a cell membrane to
regulate its energy balance. Cell membranes consist of a lipid double layer whereby the
15 lipids are made of a polar head and a fatty acid tail. Metabolic processes are regulated
by said cell membrane. Physical damage of the cell membrane may lead to inactivation
of the system or to an increase of the exchange of mass transport through the membrane
such as inter-cellular material and/or compounds present in the bulk of the product. In
the case of micro-organisms damage to the cell membrane may lead to inactivation of
20 the organism such that the cell division process will be interrupted or its functional
abilities to produce metabolic compounds is affected.

Damage to the cell membrane of micro-organisms may be caused by bringing the
micro-organisms into a high electric field. An sufficiently high externally imposed
potential difference across the micro-organism is believed to lead to damage of the cell
25 membrane as it leads to the inactivation of the micro-organisms as such. A treatment
based on PEF can performed by using a pulsed DC voltage source. The above
mentioned PEF process relies on the use of high voltage pulses to generate a pulsating
electric field of in a product of such a short duration that the heating of the bulk product
is restricted.

30 A very simple system in which the PEF-method is applied is described in
US5393541 and US5447733. Both related publications illustrate a system comprising
a container which is filled by product to be treated and a metal electrode which is
lowered into the container. The container itself forms the other electrode and both

electrodes are connected to a power supply delivering pulses of at 2 kV or more with a duration of typically two microseconds.

Another embodiment of a chamber for treating fluid products according to the PEF-method is described in US4695472 and US4838154. In this embodiment two flat
5 electrodes are positioned opposite each other with a flow channel in between. Both electrodes are connected to a power source which during operation generates pulses. In this configuration a pulsed electrical field is produced within the product inside the channel in agreement with the PEF-method. As described, in both patents the product is subjected to high electric field pulses each having a minimum field strength of at least 5
10 kV/cm and each having a duration of at least about one micro-second. Preferably a duration in the range from about 5 to about 100 micro-seconds.

A further example of a system in which a PEF-method is performed is described in US5235905, US5776529 and an article with the title "Inactivating Micro-organisms Using a Pulsed Electric Field Continuous Treatment System" by Bai-Lin Qin
15 published in IEEE Transactions on Industrial Applications, Volume 34, nr. 1, 1 February 1998, pages 43/49. This prior art system comprises a so-called coaxial treatment chamber. During operation electrical pulses are supplied to both electrodes such that electrical field strengths in the range of 35 to 55 kV/cm are developed. Preferred pulse duration's are less than 100 milliseconds, more preferably in the range
20 of 0.1 to 100 microseconds and even more preferably in the range of approximately 0.2 to 10 micro-seconds.

A system comprising a series of tubular treatment chambers is described in US5690978. Each chamber has electrically conducting end-sections, which act as electrodes separated by a non-conducting intermediate section. During operation a
25 pulsed electric field is developed in the treatment chamber with a typical pulse duration time of three microseconds at an applied electric field strength of $E = 30$ kV/cm whereas the temperature reaches a maximum $T = 36^{\circ}$ C.

In all these prior art systems the medium to be treated has to be in physical and electrical contact with both the electrodes during the treatment.

30 A different mode of treatment is described in an article with the title "Inactivation of *Yersinia enterocolitica* Gram-Negative Bacteria using high voltage pulse technique" by Piotr, Lubicki et al published as record of the industry application conference (IAS, Orlando, October 9/12, 1995, Volume 2, Number 30, pages 1338-1344, Institute of

Electrical and Electronics Engineers ISBN 0-7803-3009/9, page 1339, column 1, lines 3-24). In this article a treatment device is described comprising a cylindrical electrode system including a rod shaped inner electrode inside a cylindrically shaped outer electrode. The inner electrode is connected to a source of high voltage pulses and the
5 outer electrode is electrically grounded. The product to be treated is contained between both electrodes in a helical shaped glass tube and the remaining space within the electrode system is filled with water.

During operation a pulsed electrical field is developed between the electrodes where the rise time of each pulse is between 500 and 1300 *nanoseconds* and the voltage
10 has a peak value equal to 45, 60 or even 75 kV. The article, however, does not provide any information about the electrical field strength within the product to be treated nor the processing temperatures of the product. In the article it is stressed that "in order to cause electroporation of a cell membrane, the voltage magnitude must be high enough to induce suitable value of transmembrane potential for breakdown of the membrane,
15 and at the same time, duration of the voltage pulse must be at least higher than the relaxation time of a bacteria suspension". The product to be treated in the described model is a solution of NaCl in water for which $\epsilon = 0.7$ nF/m with an electrical conductivity between 0.8 and 1.2 S/m. The relaxation time is therefore between 0.6 and 0.9 nanoseconds. In other words, the above mentioned rise time of 500 to 1300 ns
20 is indeed significantly larger than the relaxation time of the product to be treated. It is furthermore indicated that "there is no remarkable effect of increasing rise time within the range of 500 to 1300 ns".

OBJECT OF THE INVENTION

25 An objective of the invention is now to provide another method for treating suitable products, which may contain micro-organisms by developing pulsed electrical fields within the product by a different coupling. More specific it is an objective of the invention to provide a method for mild preservation of products where direct contact between the product and the electrodes is not required and where a different
30 phenomenon is exploited to produce a substantial electrical field inside a product.

THE INVENTION

In agreement with these objectives, the invention now provides a method for

treating products by bringing the product into a treatment device containing two electrodes onto which a rapidly changing high voltage difference is imposed. The two electrodes are connected to an electronic circuitry such that the device and the product are subjected to a time dependant voltage. The time dependence of the imposed voltage is primarily characterised by the rise time of the voltage which is in duration shorter than the so-called relaxation time of the product. The relaxation time has to be understood as the time necessary to obtain a complete separation of charges in a product from the moment an external voltage difference is induced over a product column. The charges in a food product may be the result of a mineral salt content of e.g. NaCl or KCl. In foodstuffs of sufficiently high water content the NaCl molecules are dissolved as Na⁺ and Cl⁻ ions. The relaxation time can be expressed as $\tau = \epsilon/\sigma$ whereby σ is the electrical conductivity of the fluid and ϵ is the dielectric constant or permittivity.

15 Dynamical Polarisation Process

This method according to the invention, called the Dynamic Polarisation Process or DPP method, is based on the insight that foodstuffs and bio-mass in general are neither not very good conductors nor insulators. Typically, the electrical conductivity of high water content foodstuffs range from 0.1 S/m to 10 S/m and the permittivity is close to the permittivity of water i.e. 0.71 nF/m. As a result, a product column that is initially polarised by an external imposed voltage difference, will lose its polarisation after 0.07 to 7 nanoseconds. In this application this impulse response is exploited as follows: if an electrical voltage is imposed sufficiently fast by means of an external source, an electrical field will be present inside the product for a duration equal to the relaxation time. As soon as a pre-determined maximum peak amplitude is obtained, the external imposed voltage is allowed to vanish. Thus, it is only necessary to reach a maximum required voltage in order to induce a voltage gradient within a product for a certain period of time. The treatment can be applied several times by allowing more cycles as described previously. The level of the required voltage difference needed (or electrical field strength) in a particular application depends on the type of bulk medium, the micro-organisms under consideration and the number of cycles. Note, that micro-organisms that are present in the product will be affected by the voltages cycles as well. As the dynamical polarisation process is distinctly different from the PEF process, the

interaction with organisms is of a different origin. As no charge is displaced in case of DPP and the coupling to the product is capacitive the inactivation of rigid micro structures as bacterial spores may be possible as well.

It is preferred that the described DPP method is performed under circumstances whereby the trailing edge of each pulse has ended within the relaxation time of the product. Under these circumstances any electrical current due to movement of charges is prevented even if there is a physical and or electrical contact between the electrodes and the product. In general, the DPP process can be applied e.g. in continuous flow where product is pumped through a device in which treatment takes place during its residence. Product preparation, treatment and after handling of foodstuffs, pharmaceuticals etc. can be are similar to systems where heat pasteurisation/sterilisation or PEF treatment are applied in continuous flow. The exception is that treatment is employed at DPP conditions.

In case of batch operation it is preferred that there is no direct contact between the electrodes and the product. In this case the electrodes in a treatment device can be the plates of a capacitor configuration and the product just has to be present between said plates. In such a embodiment of the method, the product is not confined to a specific tube, channel etc. defined by the treatment device itself but the product is present e.g. within a suitable package which is placed into the device. Examples of products that may be treated in batch are pouches, boxes, containers but also complete eggs in shell. These products can be treated in a (semi-) continuously fashion. For this an controlled automated system may be used comprising a conveyor belt and a treatment device which is connected to suitable electronic source to apply the DPP method.

FIGURES

In the following part of this specification the invention will be described in more detail with reference to the attached drawings.

Figure 1a illustrates a first principle embodiment of a semi-continuous or batch operated device for performing the method according to the invention.

Figure 1b illustrates a second principle embodiment of a device for performing the method according to the invention in continuous flow.

Figure 2a illustrates schematically a pulse series applied in a typical PEF process.

Figure 2b illustrates schematically a pulse series applied in a process according to the invention.

DETAIL DESCRIPTION

5 The apparatus in figure 1a comprises two electrically conducting plates, 10 and 12, which together form a capacitor configuration. Both plates are connected through the respective wires 18 and 20 to a electrical power source 22. Between the plates a packed product 14 is positioned. Typically, the packaging material is made out of electrical insulating material e.g. plastic, glass or carton which contains the product to
10 be treated.

 The apparatus in figure 1b comprises two electrically conducting plates, 30 and 32, which configure a capacitor configuration. Both plates are connected through the respective wires 38 and 40 to a electrical power source 42. Between the plates a conduit 34 is installed through which the product is treated in continuous flow. Typically, the
15 conduit is made out of electrical insulating material. This conduit is part of the treatment device.

 In figure 1a a product is positioned between the plates 10, 12. The product may for instance be transported and loaded in the device by a conveyer belt 17. In figure 1b there is contact between the plates 30, 32 and the conduit 34. The conduit is part of the
20 device. It is supposed that this conduit is made of electrically insulating material. For treating the product 16 respectively 36 the source 22 respectively 42 provides high voltage pulses with a properly defined rise time and peak voltage. An example of a suitable pulse cycle is illustrated in figure 2a. The illustrated pulses are characterised by a rising edge 52, a short section 54 at maximum voltage level and a trailing edge 56.

25 If a pulse with a proper rise time is supplied to a capacitor configuration as illustrated by figure 1a or 1b an electric field is generated instantaneously within the product by means of molecular polarisation. Momentarily an electrical field is present within the product. This will induce an ion migration process within the product to be treated. Therefore, an electrical field due to DPP can exist only temporarily. After a
30 time period τ this field will be eliminated by the ion displacement. At this point the electrical field within the product is cancelled. This short period of time, also known as the relaxation time, is dependent on the electrical conductivity σ of the product and the dielectric constant ϵ according to: $\tau = \epsilon/\sigma$.

In case the externally imposed voltage pulse has a rise time which is shorter than the above-mentioned relaxation period the molecular polarisation will temporarily cause an electric field across the product and therewith across the biological cells within the product. If the strength of this induced field is sufficient, this probably leads to damaging of membranes, as is known to be present. Due to the non-stationary nature of this mechanism the time dependence has to be considered. In this situation no stationary electric current is exploited as in the case of the PEF process.

Heat dissipation

10 An amount of heat will be developed as result of dielectric losses caused by molecular rotation and ionic losses. As pointed out previously, the rise time τ_r of the imposed pulse illustrated in figure 2a has to fulfil the requirement $\tau_r < \tau$. The top section of the pulse is preferably selected very small to avoid any further migration process as soon as the maximum voltage level is reached. That implies that preferably the sum of
15 the periods τ_r and τ_p has to be smaller than τ .

Apart from the rise time, the maximum voltage level V_m obtained at the end of the rise time has to be selected as well. This level is dependent on the type of product, kind and thickness of packaging materials and the configuration of the treatment device. In general, the peak voltage should be such that a sufficiently high electrical field
20 strength is reached inside the product under consideration. Typically field strengths in excess of 1 kV/cm should be employed. The number of polarisation cycles needed and the time lag between them depends on what energy input is required and what the maximum temperature is that during the process can be allowed. In practice not one but a larger number of pulses will be needed to obtain a more intense treatment.

25 To clearly indicate the difference in the pulse characteristics used in the PEF process on the one hand, and the process according to underlying application on the other hand figure 2b is added. On the same time axis a typical PEF pulse and a typical DPP pulse are illustrated for the treatment of an assumed high water content product with a electrical conductivity of 1 S/m. The following values are typically required:

	PEF	DDP
rising edge	100 nsec	0.7 nsec
steady state	2000 nsec	<0.7 nsec
trailing edge	100 nsec	<0.7 nsec.
maximum imposed electrical		
field strenght (inside product)	30 kV	30 KV

DPP in practice

In a practical test the method discussed in this application was exercised by stopping the lactic acid production by Lacto bacilli strains. In foodstuffs this kind of organisms are known to cause spoil food during storage by acidification, thereby limiting the shelf life during storage. A small amount of fresh yoghurt, dissolved in demineralised water, was used as a model product. The inoculation of the samples was approximately 10^5 organisms per millilitre at a pH=7.0. The conductivity of the inoculated buffer was such that a relaxation time in excess of 100 ns was obtained for one part of the batch and a relaxation time of much less than 100 ns for a second part of the batch. The two different stocks of model product were distributed over 64 bags, made out of plastic film bags (Stomach). The tickness of the bags was 100 micrometer and the permittivity of the material is 270 nF/m. The bags were hermetically sealed allowing a minimal head space of air as a result of the sealing procedure. Part of this batch was non-treated as a reference, a second part was treated by conventional heat (pasteurised) at 80 degrees centigrade for 10 minutes. A third part of the batch was treated at different DPP conditions and at intensity levels. This included the cases where either the conditions where $\epsilon/\sigma > \text{pulse rise time}$ and $\epsilon/\sigma < \text{pulse rise time}$. In other words, several different control experiments have been taken into account to evaluate the validity of the claims in this patent application.

A custom-made high voltage power supply was used to produce voltages cycles over more than 15 kV within 100 ns. In the demonstration set-up the film bags where provided with adhesive aluminium foil at the outer side. The film bags loaded into a device similair as described in figure 1a. Note that as the bags are made out of plastic, which is an good electric insulator, no charge can flow through the product.

After treatment the samples were added to a sterile sample of milk at pH 7.0 which were incubated at 40 degrees centigrade for 12 hours hereafter. After this time,

the pH of the samples was measured to check whether acidification had occurred or not. A reading of $\text{pH} < 5.0$ was considered as acidified or spoiled whereas levels of $\text{pH} > 6.8$ were considered as non-spoiled. The result of these treatments can be found in table 2. In case of the non-treated samples all of the 17 samples demonstrated spoilage.

5 For the chosen level of pasteurisation 16 bags out of 23 were successfully heat pasteurised. For 7 bags spoilage could be demonstrated indicating that some Lacto Bacilli survived pasteurisation at this temperature-time combination. For the DPP processed bags the electric field strength of the treatment have to be sufficient intense as well as the total treatment time. The total treatment time is defined by the relaxation

10 time multiplied by the number of voltage cycles employed. Furthermore, the necessity of the required condition that $\epsilon/\sigma > \text{pulse rise time}$ is demonstrated. In treatment G in table 2, the proper conditions were found to completely stop the acidification. In this case 8 out of 8 treatments has led to a full suppression of the spoilage. This was achieved at a maximum product temperature of 40 degrees centigrade. In addition, the

15 total processing time needed is much less than the required time for heat pasteurisation at 80 degrees centigrade.

As shown in table 1, in all cases the temperature increase was restricted to small values which in general are not obtainable with prior art methods.

20 Table 1 Measured temperature increments for various treatments. The ambient temperature was 20 degrees centigrade.

	Treatment	Treatment Period	Field strength (kV/cm)*	Temperature Increase (°C)
		(ms)		
25	A	2	20	0.5
	B	20	20	1.0
	C	200	20	8.5
	D	200	25	18.0

30

(*) Within the product. This value is due to the instantaneous polarisation (DPP process) and is determined by finite element analysis modelling of the total system. The field strength is evaluated at the maximum applied voltage.

In table 2 the inactivation of Lacto Bacilli in sealed bags and treated with the DPP process is compared with a conventional heat treatment:

5	Table 2				
	Treatment (time)	relaxation time of product	E (kV/cm)*	number of bags	% acidified
	None	< > 100 ns	-	17	100%
	heat pasteurisation	< > 100 ns	-	23	30%
10	E (2 ms)	< > 100 ns	20	4	100%
	F (200 ms)	< > 100 ns	13	12	100%
	G (200 ms)	> 100 ns	20	8	0%

(*) Within the product. This value is due to the instantaneous polarisation (DPP process) and is determined by finite element analysis modelling of the total system. The field strength is evaluated at the maximum applied voltage.

Characteristics

In the following the typical characteristics of both PEF and DPP are shortly resumed:

In a typical PEF process pulses of the type illustrated in figure 2b are applied. During a PEF-treatment the product to be treated is in contact with two conducting electrodes which are connected to a pulsed power source. By means of said power source an stationary electric field is imposed according to $j = \sigma E$. Typically the pulses, applied to said electrodes are maintained for some microseconds. Under these conditions a stationary situation is obtained in terms of the electric parameters current and voltage. That is an electric current density by movement of charge is required to sustain a net electric field. This is by transport of e.g. ions dissolved in the product which are dragged to the product during the actual treatment.

One or more of the beneath indicated characteristics are typical for PEF-treatment:

(1) there is a continuous supply of electric current during the pulse. This current is delivered by a capacitor in an electronic pulse circuit whereby a so-called pulse-

forming network (PFN) may be used to maintain the voltage across the treatment device constant while the discharging the capacitor. If no PFN is applied the voltage will decay exponentially in time. The time constant RC of this decay is related to the capacity (C) of the charged capacitor and to the ohmic resistance R of the electronic circuit:

(2) the electronic circuit supplies a peak power which can be related to $P = \sigma E^2 V$

whereby E is the spatial average of the field strength applied, σ the electric conductivity of the product and V is the total volume of the product being treated;

(3) the pulse is preferably of rectangular shape, in other words: the voltage is kept to a constant level during the duration of the pulse;

(4) a typical pulse duration of some microseconds is applied;

(5) the electrodes are in physical contact with the medium to be treated;

(6) the average dissipated power $\langle P \rangle$ is give by $\langle P \rangle = \sigma E^2 t j$ whereby t is the total treatment time and j is the product throughput.

(7) the heat development in the PEF process is determined by Ohmic heating and is given by $\langle P \rangle = \langle U \rangle \langle I \rangle$, wherein $\langle U \rangle$ and $\langle I \rangle$ are respectively the time averaged voltage and the time averaged electric current of the imposed pulse shape. The total current as such is determined by the current density $j = \sigma E$ and the cross sectional area of the surface through which said current is measured.

For a description of the Dynamic Polarisation Process (DPP) according to the invention, the above-mentioned simplification that the current density goes beyond the time-independent, stationary state approximation given by $J = \sigma E$. Instead, we have to take into account all of the so-called Maxwell equations and allow time dependent polarisation effects. In this case the current density is given by:

$$j = \sigma E + dD/dt$$

where the dD/dt term is the time derivative of the molecular displacement field. D is related to the static polarisation vector of a dielectric medium with permittivity ϵ , by:

$$D = \epsilon E.$$

In the above indicated applications of systems based on PEF treatment the second term

in the equation is considered to be zero. At the pulse conditions where PEF systems operate in practise this is a valid assumption. However, in underlying application use is made of the action relying by the second term, i.e. dD/dt . It can be proven that with rapidly varying voltages through a capacitive coupling only the second term of the
 5 above mentioned equation is significant for a predetermined microbial effect. In contrast to the conducting current in the PEF-process, the displacement current in underlying application is only a reactive current which is present only temporarily after the initial polarisation of the medium.

The method according to the invention is now characterised by a number of the
 10 hereafter indicated characteristics:

1. the change of an the external imposed voltage generates an electrical field between two-capacitor plates which causes an temporarily voltage gradient across the product and across any present membrane structure;
2. the maximum of the imposed voltage should be sufficiently high to cause micro-
 15 biological inactivation. Typically field strengths are employed in excess of 1kV/cm.
3. the external voltage has to imposed within a time interval smaller than the dielectrical relaxation time of the product given by its electrical conductivity and permittivity by $\tau = \epsilon / \sigma$,
4. the heat that is disposed in the product as a result of the polarisation-
 20 depolarisation cycles determined by the number of times the medium is polarised. In general, the energy density in a polarised medium is given by $u = 1/2 \epsilon E^2$. After each cycle of polarisation and depolarisation this equals the maximum energy contents which can be converted into heat. In case that a continuous wave (CW) oscillator of frequency ω is facilitated as the external voltage source, the heat dissipation per unit
 25 volume is determined by:

$$p = 1/2 \omega \epsilon \epsilon'' E^2, \text{ where } \epsilon'' \text{ is the so-called dielectrical loss factor.}$$

5. the heat development is caused primarily by the counter acted rotation (friction) of
 30 polar molecules and ions in the medium to be treated. For radio frequencies (RF) in the range of 1 to 1000 MHz these kind of losses are relatively small, for microwave (MW) frequencies in the range of 1-4 GHz these losses can be significantly.

By applying the method according to the invention products containing micro-

organisms and membrane structures can be treated in a completely different manner. It has been demonstrated that this method in principle can be used to stop bacterial spoilage at reduced temperatures and treatment times. An example of the performance of this novel method has been demonstrated and has been compared to a standard heat process (pasteurisation) that is commonly used by food manufacturers and the pharmaceutical industry as a preservation method. The novel method can be applied as a mild preservation method for pre-packed products and has potential as a mild decontamination method of in shell eggs. Although this method has been evaluated for a pre-packed product, it is also applicable as a continuous process on a flow of bulk produce. To generalise its application: the method relies on dynamical polarisation cycles, induced by means of an external time dependant voltage source with specific requirements on the steepness of the rising edge of the imposed voltage shape rather than the duration of the imposed voltage. The basic differences between the dynamic polarisation processes according to underlying application and the prior art PEF-method are:

- use is made of a non-stationary electronic processes to generate an electrical voltage difference across a product;
- the coupling with the medium to be treated is capacitive;
- in principle no electrodes in physical contact with the product are required.

In contrast to the PEF-technology, the process according to underlying application functions in principle with cycles of continuously oscillating electrical fields. These can be applied by using electronic circuitry operating at a single resonant frequency. This is fundamentally different from the types of electronic circuitry employed in the PEF technology, where switches and PFN's have to be operated over a spectrum of frequencies to obtain an electrical pulse.

CLAIMS

1. Method for treating products, which may contain cellular material of eukaryotic and/or prokaryotic origin and in particular micro-organisms, by bringing the product in device comprising two electrodes connected to an electronic circuit such that in said
5 device and in said product a pulsating electrical field is created, characterised by the rise time of each imposed voltage pulse which is less than the electronic relaxation time of the product.
2. Method for treating products according to claim 1, where the cellular structures
10 present in the product are pathogenic or spoilage organisms and or there spores, where treatment is applied as a method to prevent the outgrowth of these organisms in the product after production during distribution or storage.
3. Method for treating products according to claim 1, where the cellular structures
15 present or the bulk product contains certain compounds which are exchanged at a higher rate through the membrane of such structures during or after the treatment.
4. Method for treating products according to claim 1, characterised in that the rising
20 edge of each cycle starts within the relaxation time of the product.
5. Method for treating products according to claim 1 or 2, characterised in that each electrical field pulse has a duration shorter than the relaxation time of the product.
6. Method according to any of the preceding claims, characterised in that dependent
25 on the type of product and the types of micro-organisms contained in the product, the maximum fieldstrength of each pulse, the repetition frequency and the number of cycles in the treatment are selected such that the target micro-organisms and or spores are functionally inactivated leading to into a shelf life stable, microbiologically safe product.
7. Method according to any of the preceding claims, characterised in that dependent
30 on the type of product, the types of cellular structures contained in the product, the maximum field strength of each pulse, the repetition frequency and the number of

cycles during the treatment are selected such that the target cells are functionally affected, not necessarily inactivated, leading to an enhanced exchange of intracellular compounds or compounds present in the bulk through the membrane structures of the cells.

5

8. Method according to one of the preceding claims, where the imposed voltage on the treatment device is produced at a single frequency or at multiple, distinct frequencies using electronic resonant structures or circuitry at frequencies in the range of 1 kHz to 10 GHz.

10

9. Method according to any of the preceding claims, characterised in that the maximum field strength of each pulse, the repetition frequency and the number of cycles during the treatment are selected such that the temperature of the product does not exceed a predetermined value during treatment.

15

10. Method according to one of the preceding claims, characterised in that the temperature of the product is maintained at temperatures lower than required by conventional heat based processes, and or characterised by a treatment time less than required in a conventional process at the same temperature.

20

Fig 1a

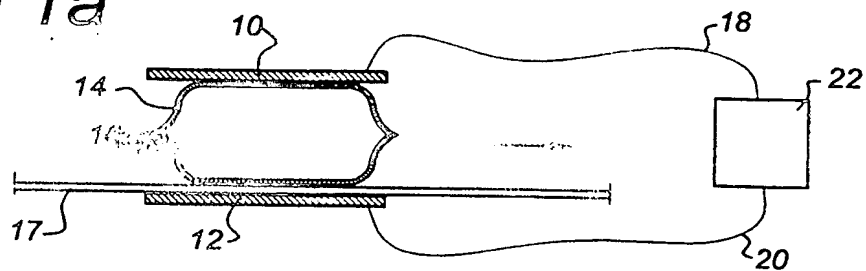


Fig 1b

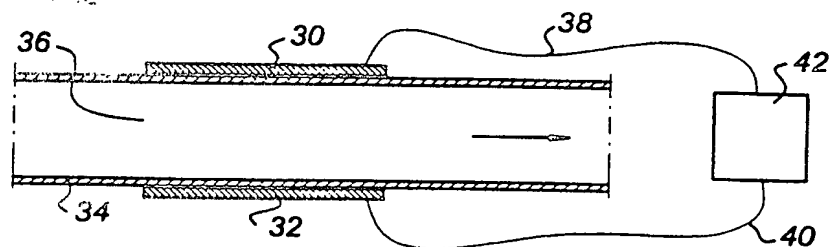


Fig 2a

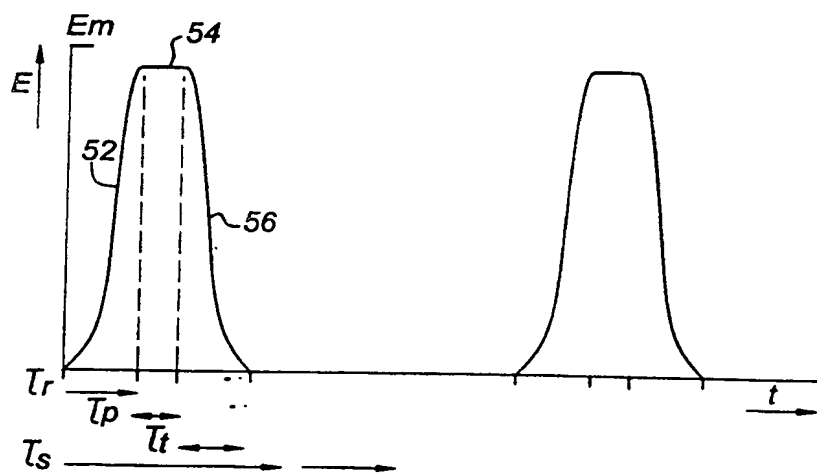
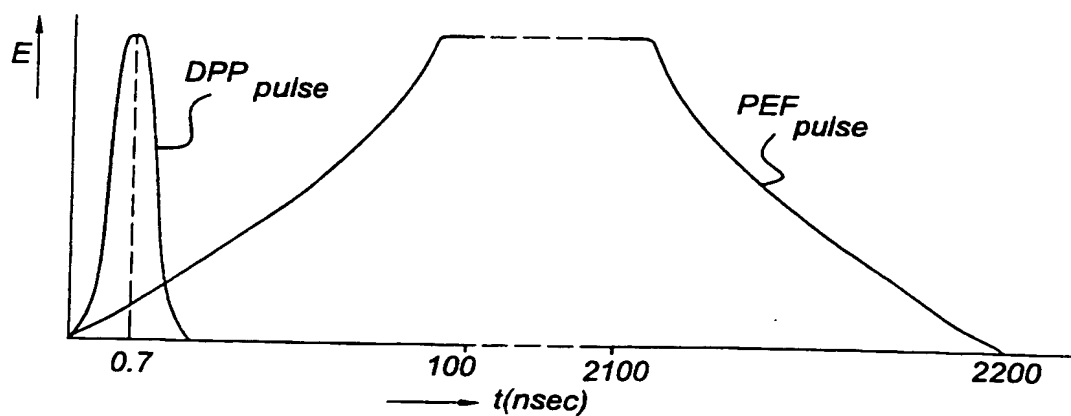


Fig 2b



INTERNATIONAL SEARCH REPORT

International Application No.
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23L3/32 A23B5/015

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L A23B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, PAJ, EPO-Internal

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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
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- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

Int. l. Application No
PCT/NL 00/00200

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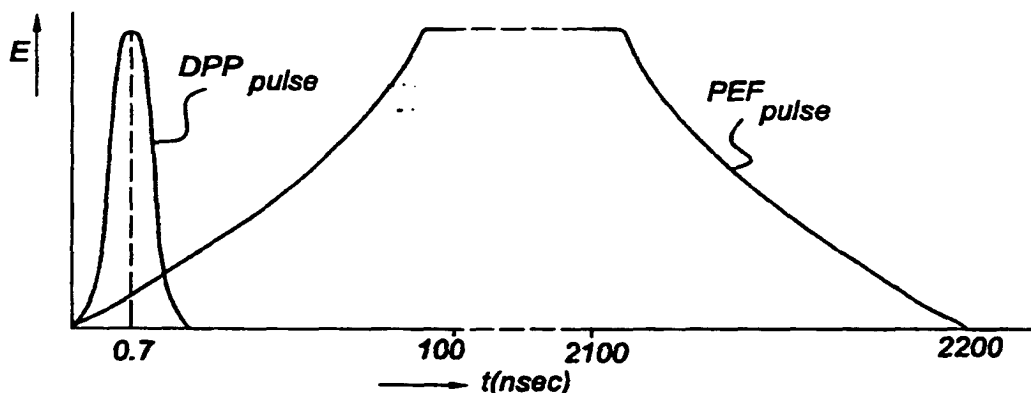
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- (30) Priority Data:
1011660 24 March 1999 (24.03.1999) NL
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- (54) Agent: JORRITSMA, Ruurd; Nederlandsch Octrooibureau, Scheveningsweg 82, P.O. Box 29720, NL-2502 LS The Hague (NL).
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- Published:
— With international search report.
— With amended claims.
- Date of publication of the amended claims: 7 December 2000

[Continued on next page]

(54) Title: METHOD FOR TREATING PRODUCTS BY HIGH VOLTAGE PULSES



(57) Abstract: The invention relates to a method for treating products, which may contain cellular material of eukaryotic and/or prokaryotic origin and in particular micro-organisms, by bringing the product in device comprising two electrodes connected to an electronic circuit such that in said device and in said product a pulsating electrical field is created, characterised by the rise time of each imposed voltage pulse which is less than the electronic relaxation time of the product. Preferably the rising edge of each cycle starts within the relaxation time of the product and even more preferably each electrical field pulse has a duration shorter than the relaxation time of the product. Dependent on the type of product and the types of micro-organisms contained in the product, the maximum fieldstrength of each pulse, the repetition frequency and the number of cycles in the treatment are selected such that the target micro-organisms and/or spores are functionally inactivated leading to a shelf stable, microbiologically safe product.

WO 00/56179 A1

WO 00/56179 A1



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

AMENDED CLAIMS

[received by the International Bureau on 18 September 2000 (18.09.00);
original claims 1-10 replaced by amended claims 1-11 (2 pages)]

1. Method for treating products, which may contain cellular material of eukaryotic and/or prokaryotic origin and in particular micro-organisms, by bringing the product in a treatment device comprising two electrodes connected to an electronic circuit such that in said device and in said product an electrical field in excess of 500 V/cm is created, provided that the rise time or the trailing edge of each imposed voltage pulse is shorter than the associated electronic relaxation time of the product defined by the electrical properties of the product.
2. Method for treating products according to claim 1, where the cellular structures present in the bulk product are pathogenic or spoilage organisms and/or their spores, where treatment is applied as a mild preservation method to prevent the outgrowth of such organisms in the product after production during distribution or storage.
3. Method for treating products according to claim 1, where the cellular structures present or the bulk product contains certain compounds which are exchanged at a higher rate through the membrane of such structures when applying the treatment.
4. Method for treating products according to claim 1, characterised in that the trailing edge of each pulse starts within a time span shorter than the relaxation time of the product.
5. Method for treating products according to claim 1 or 2, characterised in that each electrical field pulse has a duration shorter than the relaxation time of the product.
6. Method according to any of the preceding claims, characterised in that dependent on the type of product and the types of micro-organisms contained in the product, the maximum field strength during a cycle, the repetition frequency and the number of cycles during a treatment are selected such that the target micro-organisms and or spores are functionally affected or inactivated leading to into a shelf life stable, microbiologically safe product.

7. Method according to any of the preceding claims, characterised in that dependent on the type of product and the types of cellular structures contained in the product, the maximum field strength reached in a cycle, the repetition frequency and the number of cycles during a treatment are selected such that the target cells are functionally affected, not necessarily inactivated, leading to an enhanced exchange of intracellular compounds with the bulk product.
8. Method according to one of the preceding claims, where the imposed voltage on the treatment device is produced at a single frequency or at multiple distinct frequencies using electronic circuitry at frequencies in the range of 100 Hz to 10 GHz.
9. Method according to one of the preceding claims, where the imposed voltage on the treatment device is produced at a single frequency or at multiple distinct frequencies using resonant electronic structures at frequencies in the range of 100 Hz to 10 GHz.
10. Method according to any of the preceding claims, characterised in that the maximum field strength of each pulse, the repetition frequency, the number of cycles and the total residence time during a treatment are selected such that the temperature of the product does not exceed a predetermined value during treatment.
11. Method according to any of the preceding claims, characterised in that the temperature of the product is maintained below 121 degrees centigrade.